Cat.#R1004 *Kwik*Quant Western Blot Detection Kit

Kit Components:

1. Digital Anti-mouse IgG-HRP (1mL)
2. Digital Anti-Rabbit IgG-HRP (1mL)
3. *Kwik*Quant Ultra HRP Substrate Solutions (50mL A solution + 50 mL B solution)

Procedures

1. Blocking: in 5% skim milk in 1x *Clean*Western buffer at room temperature for at least 1 hour.

2. Rinse the membrane briefly using water to get rid of excess milk.

3. Dilute your primary antibody in *Stamina* Primary Antibody Dilution Buffer (cat# R2004) and probe the membrane **at room temperature** for 1-24 hours (method 1)

(or in 5% skim milk in 1x *Clean*Western Buffer in at 4°C for overnight, method 2).

Sensitivity of method 1 is at least twice as that of method 2.

4. Recover the primary antibody solution and store it at 4°C. The antibody is stable in the solution, and can be reused many times without activity loss.

5. Wash the membrane using 1x *Clean*Western Buffer three times, 15mL x 10min each.

6. Dilute (@1:1000) the Digital HRP-secondary antibody in 1x *Clean*Western Buffer with 5% skim milk. Probe the membrane with rocking at room temperature for 15 minutes ~ 4 hours. Longer incubation time will significantly increase detection sensitivity.

7. Wash the membrane using 1x *Clean*Western Buffer three times, 20mL x 10min each.

8. Rinse the blot with de-ionized water* briefly to remove the residual *Clean*Western Buffer, and transfer the blot onto the sample plate of *Kwik*Quant Imager. (*The buffer contains reagents that will

inhibit the reaction of HRP-ECL substrate.)

9. Submerge the membrane in 1:5 H_2O-diluted *Kwik*Quant Digital-ECL mixture (A:B=1:1) for 2 minutes.

10. Put the membrane on a *Kwik*Quant Imager (or any chemiluminescence imagers) for image acquisition.

11. If the bands are too weak at 2 min exposure, submerge the membrane in non-diluted *Kwik*Quant Digital-ECL mixture (A:B=1:1) for another 2 minutes, followed by image acquisition.